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13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information) In the proposed studies, novel T cell immunotherapies against breast cancer will be developed based on studies demonstrating a positive correlation between T lymphocytic infiltration of these tumors and favorable clinical outcome. The major goal of the proposed studies is to isolate and characterize cytolytic T lymphocytes (CTL) with <i>in vivo</i> -like T cell receptors. The CTL provide the basis for adoptive CTL immunotherapy and active immunotherapy with CTL-derived peptides/antigens. During the past 3 months of study approved for inclusion of human subjects, 4 breast carcinoma tissues were cultured in organotypic cultures (reconstructs) and mixed lymphocyte/tumor cultures (MLTC). Eleven T cell lines were obtained from 2 breast cancer specimens and 3 fibroblast cell lines from 3 specimens. Our preliminary studies also have shown that breast tumor cells grow <i>in vitro</i> (reconstruct and MLTC), although it is too early to determine the success rate for establishing long term tumor cell lines. These preliminary studies demonstrate the feasibility of establishing T cell lines against breast cancer cells in a novel culture system with <i>in vivo</i> relevance (reconstruct).			
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INTRODUCTION

The presence of a T cell infiltrate has been associated with a favorable prognosis in patients with breast cancer (1-3). These studies suggest the feasibility of adoptive breast cancer immunotherapy with cytolytic T lymphocytes (CTL) or active immunotherapy with CTL-defined antigens. To develop such therapies, CTL and tumor cells are traditionally isolated and cultured directly on plastic surfaces in a 2-dimensional mixed lymphocyte/tumor cell culture (MLTC), and tumor-specific CTL lines or clones are isolated. However, immunotherapy trials with adoptive CTL transfer and active immunotherapy with CTL peptides in breast cancer patients have not held promise (4-8). The failure of these therapies might be explained by the marked differences between the CTL generated in ML TC *in vitro* compared to the CTL under *in vivo* conditions, as suggested by studies conducted in melanoma patients (9-11). Thus, we are testing the hypothesis that changes in the T cell receptor (TCR) repertoire occur upon culture of breast carcinoma tissues in ML TC *in vitro*, which might explain the absence of clinical responses in adoptive and active immunotherapy of breast cancer patients. In the proposed studies, CTL are generated under tissue-like conditions in an organotypic breast carcinoma culture system (reconstruct), so that the *in vivo* phenotypic and functional characteristics of the cells are preserved as much as possible.

BODY

The specific objectives of this proposal are to:

1. Isolate T cells and tumor cells from fresh, uncultured breast carcinoma tissues, or tissues cultured in the reconstruct or MLTC.
2. Compare phenotype and function of T cells isolated from fresh, uncultured breast cancer tissue, or tissue cultured in reconstruct or MLTC.
3. Demonstrate CTL migration toward tumor cells.

We obtained Human Subject and Human Anatomical Substance Approvals from USAMRAA in July 2004. Thus, the proposed studies were initiated in July 2004 and this report covers the period between July 2004 and October 2004. Progress has been made on specific Aim 1.

Establishment of breast carcinoma and T cell lines from fresh tumor tissues

Primary breast carcinoma tissues and heparinized blood from 4 patients were obtained from our collaborator Dr. Eric Miller (Virtua Memorial Hospital, NJ). A summary of patients' characteristics is included in Table 1. The tissues were minced, and one part was cryopreserved for T cell characterization. The second part served to initiate T cell lines in reconstructs with bovine type I collagen, and in ML TC directly on plastic surfaces. The third part was used to establish long-term tumor and fibroblast cell lines directly in plastic dishes. Minced tumor tissue was also injected into SCID mice to obtain long-term tumor cell lines. Peripheral blood mononuclear cells

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were separated from blood specimens by Ficoll-Hypaque density centrifugation, and cryopreserved for later use as antigen-presenting cells.

Table 1. Summary of subjects studied

Patient number	Breast cancer tissue obtained	Patient		
		Age	Gender	Ethnic background
BCP-01	Primary lesion	44	Female	Caucasian
BCP-02	Primary lesion	54	Female	Caucasian
B-3	Primary lesion	63	Female	Caucasian
B-4	Primary lesion	57	Female	Caucasian

From one tissue (BCP 01), 6 T cell lines were established from both MLTC and reconstruct (22×10^6 cells cryopreserved; Table 2). From another specimen (BCP 02) 5 T cell lines were obtained from reconstruct only (16×10^6 cells cryopreserved; Table 2). Most likely, T cell lines will also be established from the 2 recently obtained tissues since lymphocytes are growing in both MLTC and reconstruct cultures initiated with these tissues.

Tumor cell colonies are growing in all cultures; however, it is too early to determine our success rate for establishing long-term breast cancer cell lines.

Peripheral blood mononuclear cells (PBMC) were cryopreserved from all 4 patients. These cells will be used as antigen-presenting cells.

Fibroblast cell lines have been established from 3 specimens (Table 2).

Table 2. Reconstructs (REC) and MLTC with fresh breast cancer tissues^a

Patient #/date of specimen	Number of frozen vials					
	Tumor tissue	PBMC ^c	Cell lines			
			Tumor	Fibro- blasts	T cells	
					MLTC ^d	REC ^d
BCP 01 7/17/04	6	8	? ^b	6	4	7
BCP 02 7/23/04	5	7	? ^b	2	0	8
B-3 8/19/04	7	9	? ^b	2		
B-4 9/23/04	6	10	? ^b			

^aReconstructs were initiated by seeding minced tissue into bovine type I collagen in minimal essential medium (MEM) supplemented with 10% human AB serum; 7 days later and then weekly, cultures received RPMI 1640 supplemented with 10% human AB serum and 10% natural IL-2. MLTC were initiated by seeding minced tissues in RPMI 1640 medium supplemented with 10% human AB serum into plastic culture dishes; 7 days later and then weekly, cultures received 10% natural IL-2-containing medium (RPMI 1640 plus 10% human AB serum).

^c 1×10^7 /vial

^d 2×10^6 /vial

?^b = too early to be determined

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KEY RESEARCH ACCOMPLISHMENTS

- Cryopreservation of 4 breast cancer tissues and PBMC from the same patients.
- Establishment of reconstructs and MLTC from 4 breast cancer tissues.
- Isolation of T cell lines from both MLTC and reconstruct (one tissue) and reconstruct only (another tissue).
- Determination of lymphocyte growth in reconstruct and MLTC (2 tissues).
- Determination of tumor cell growth in all reconstruct and MLTC cultures derived from 4 tumor tissues.
- Establishment of fibroblast cell lines (3 tissues).

REPORTABLE OUTCOMES

This study generated 11 T cell lines from 2 breast cancer tissues and 3 fibroblast cell lines from 3 breast cancer tissues.

CONCLUSIONS


This preliminary report covers approximately 3 months of research aimed at developing *in vivo*-like CTL against breast cancer cells. During this time period, the feasibility of the proposed studies has been demonstrated as we have successfully established 11 T cell lines from 2 breast cancer tissues. In the 2 additional tissues studied, it is too early to determine the success rate of T cell line establishment. Our preliminary studies also have shown that breast tumor cells grow *in vitro*, although it is too early to determine the success rate for establishing long-term tumor cell lines.

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